Rev. Biol. Trop., 47 (Supl. 1): 19-25, 1999
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Bacterioplankton from cenotes and anchialine caves of Quintana Roo, Yucatan Peninsula, Mexico

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Received 14-I-1999. Corrected 12-III-1999. Accepted 12-IV-1999.

Abstract. The seasonal fluctuation of bacterioplankton densities were evaluated in five cenotes and two anchialine caves of Quintana Roo, northeastern Yucatan Peninsula. Bacterioplankton densities were extremely low ($5.8 \pm 0.35 \times 10^2$ - $8 \pm 0.48 \times 10^3$ cells/ml), even for oligotrophic environments. Non seasonal differences were detected in the cenotes, however, in the caves bacterial counts were higher in the rainy season. Bacterial densities were lower in the cenotes ($5.8 \pm 0.35 \times 10^2$ - $4.3 \pm 0.26 \times 10^3$ cells/ml), and higher in the caves ($2.7 \pm 0.16 \times 10^3$ - $8 \pm 0.48 \times 10^3$ cells/ml). Rainwater percolation, rich in forest´soil particulate (POM) and dissolved organic (DOM) matter into the caves, seems to promote higher bacterial densities. In addition, surface bacteria entering groundwater in the same way as POM and DOM could also be augmenting bacterial densities in the caves. Low groundwater nutrient (0.05 - 1.7 µM total P, 34.89 - 250.9 µM total N) concentrations as well as reduce bacterial densities characterizes the cenotes and caves as ultra- to oligotrophic environments.

Key words: sinkholes, submersed caves, bacteria, plankton, nutrients.

The Yucatan Peninsula, located in the southeastern Mexico, is a limestone plain with a significant proportion of evaporites. Nearly the entire peninsula is underlain by porous and fissured limestone with a veneer of soil and xerophytes. The southernmost part is covered by a typical tropical rain forest; temperature variations are small, and seasonality is, therefore, defined by the rainy/dry season. Quintana Roo, at the eastern part of the Yucatan Peninsula, is characterized by two climatic periods that last six months each; the rainy season is from March/April to October/November, and the dry season is from October/November to March/April. Few surface-water bodies exist, and the rivers are short. Water flow occurs primarily underground (Lesser & Weidie 1988).

The Yucatan Peninsula is underlain by extremely permeable carbonate rocks that have been eroded to conform an intricate underground water-drainage system. Typical karst topography shows two different systems: 1) well-illuminated, open-water pools (sinkholes or "cenotes"), and 2) submerged caves in total darkness. Between them, the cavern conforms a transitional (twilight) zone, where both systems interact through exporting organic matter; the major input of photosynthethic organic matter (terrestrial and/or aquatic) from pools into caves, and chemosynthetic organic matter from anchialine caves into the pools (Culver 1994).

In karst -limestone- systems phosphorous is precipitated along with calcium (Margalef 1983), thus inhibiting bacterial productivity.

Nutrient supply through pollution, soil erosion, plant fertilizers, etc., could encourage bacterial productivity of the aquatic systems. Although, scant information is available regarding groundwater microbiology, the role of microorganisms in groundwater seems to be quite important. There is a close relationship between microbial activity and speleogenesis (Brigmon *et al.* 1994, López-Adrián & Herrera-Silveira 1994, Martin *et al.* 1995). *Thiothrix*, *Desulfovibrio* and other chemoautotrophic bacteria produce sulfuric and nitric acid which increase limestone dissolution. *Thiothrix* and *Beggiatoa* participated in the creation of Cesspool cave, Virginia, through sulphur oxidation. Nitrifying and denitrifying bacteria could also altered groundwater nitrate concentration (Stoessell *et al.* 1993, Brigmon *et al.* 1994).

In spite of this information, the actual knowledge regarding the bacterioplankton of karstic systems and their role therein is sparse (Edler & Dodds 1992, Brigmon *et al.* 1994). So, the aim of this paper is to evaluate bacterioplankton densities of five cenotes and two anchialine caves from the northeastern portion of Quintana Roo, Mexico, and their seasonal fluctuation. Since bacteria play a main role in nutrient cycling, also nutrients (N, P, Si) concentration were measured to correlate with bacterioplankton densities.

MATERIALS AND METHODS

The five cenotes that were studied are Carwash, Cristal, Mayan Blue, the main entrance of Nohoch and Casa. The anchialine caves associated to the Mayan Blue and Cristal cenotes were studied as well. Position of the sampling locations were determined using a Magellan Field Pro V GPS (Global Positioning System) instrument calibrated at Puerto Aventuras. Nohoch cenote is cave-like, and the other four are well-like. General characteristics of the five cenotes studied are provided on Table 1.

To detect maximum possible variations during the dry/wet periods, sampling was conducted at the end of the dry season (March, 1995), when maximum concentrations were expected, and at the end of the rainy season (October/November, 1995), when maximum dilution was anticipated. Temperature, conductivity, and dissolved oxygen vertical profiles were measured *in situ* (Hydrolab Datasonde3/Surveyor3 multiparameter water-quality datalogger and logging system) for possible stratifications (thermo, halo and/or oxyclines). When the water column was homogeneous (i.e., in the cenotes Mayan Blue, Cristal, Nohoch and Carwash) a mid-depth sample was collected with a 5L Van Dorn water bottle for further chemical and microbiological analyses; otherwise (Casa cenote), three samples were taken, one at each stratum (above -epicline-, below -hypocline-, and at the halocline). Sampling in anchialine caves was carried out *in situ* by cave divers at each one of the three strata (epicline, halocline, hypocline) with 75 ml glass (bacterioplankton) and 500 ml plastic bottles (nutrients).

For bacterial density, the sample was fixed with formalin to reach a final concentration of 2%. At the Lab, samples were stained with DAPI (4',6'-diamino-2-phenylindole), and 30 ml filtered onto black-prestained 0.2µm pore-size Millipore membrane filters (Porter & Feig 1980). Bacteria were counted using a Zeiss epifluorescence microscope under 1 250X. To assure statistical significance, minimum bacterial count reached 1 000 to attained 6% mean confidence interval (Wetzel & Likens 1979).

Samples for nutrient analysis (total N, organic N, NH_4, NO_2, NO_3, total P, PO_4, Si) were ice-cold preserved until evaluated at the Laboratory following the protocols described by Strickland and Parsons (1972), Parsons *et al.* (1984), and Stirling (1985).

Bacterioplankton densities were statistically compared (non-parametric U-Mann Whitney test), and correlation with nutrients tested (Spearman non-parametric rank correlation coefficient) (Statgraphics 5.0, 1991).

TABLE 1

General characteristics of the studied cenotes. (mbsl = meters below surface level).

	Carwash	Cristal	Mayan Blue	Nohoch	Casa
Geographic	20°16.48'N	20°12.50'N	20°11.61'N	20°17.93'N	20°15.97'N
Location	87°29.74"W	87°28.98'W	87°29.74'W	87°24.20'W	87°23.41'W
Surface Area	300 m^2	135 m^2	500 m^2	250 m^2	500 m^2
Maximum Depth	6 m	5 m	5 m	7 m	7 m
Water Level	0 mbsl	0 mbsl	0-3 mbsl	0-4 mbsl	0 mbsl
Bottom Type	Rocky	Muddy	Rocky	Rocky	Rocky with detritus
Aquatic Vegetation	Scarce	*Cabomba,* Benthic algae	*Nymphaea, Sagittaria*	Scarce	Mangroves

RESULTS

Bacterioplankton densities in both the cenotes and caves were low (5.8 ± 0.35 x 10^2 - 8 ± 0.48 x 10^3 cells/ml). In the cenotes, the highest densities were found in the dry season (5.8 ± 0.35 x 10^2 - 4.3 ± 0.26 x 10^3 cells/ml), and the lowest in the rainy season (5.8 ± 0.35 x 10^2 - 3.2 ± 0.19 x 10^3 cells/ml) (Fig. 1). On the opposite, the highest densities in the caves were found in the rainy season (5 - 8 x 10^3 cells/ml), and the lowest in the dry season (2.7 - 5.1 x 10^3 cells/ml) (Fig. 2).

Bacterioplankton counts in the cenotes ranged from 5.8 ± 0.35 x 10^2 to 4.3 ± 0.26 x 10^3 cells/ml (Fig. 1). The highest densities were found in Mayan Blue cenote in both seasons (4.3 ± 0.26 x 10^3 and 3.2 ± 0.19 x 10^3 cells/ml in the dry and rainy seasons, respectively), meanwhile the lowest densities were measured in the epicline of the Casa cenote at both seasons (5.8 ± 0.35 x 10^2 cells/ml). In spite of an apparent seasonal behavior, there were non-significant differences (U-Mann Whitney p = 0.44) between the two seasons.

Bacterial densities in the caves ranged from 2.7 ± 0.16 x 10^3 cells/ml to 8 ± 0.48 x 10^3 cells/ml (Fig. 2). In general, cave bacterial densities were higher at the epicline and lower at the halocline. In both seasons, lowest densities were measured at the Cristal cave halocline (2.7 ± 0.16 x 10^3 cells/ml and 5 ± 0.3 x 10^3 cells/ml in dry and rainy seasons, respectively), and the highest at the epicline of the Mayan Blue cave (5.1 ± 0.31 x 10^3 cells/ml and 8 ± 0.48 x 10^3 cells/ml in dry and rainy seasons, respectively). There were significant differences tested (U-Mann Whitney p = 0.008) between cave bacterial densities in the rainy and the dry seasons being higher in the former.

A statistical comparison showed significant differences (U-Mann Whitney p = 0.0004) between bacterial counts of cenotes and caves, being higher in the latter (2.7 - 8 x 10^3 cells/ml) and lower in the former (5.8 x 10^2 - 4.3 x 10^3 cells/ml).

Nutrient concentrations are low. Ammonia, although in low concentrations (1.47 - 8.32 µM) is the second most abundant form of nitrogen after the nitrates. The nitrite concentrations (not detected-0.87µM) are insignificant compared with the other nitrogen species. The most abundant nutrient is nitrogen in form of nitrate (4.14 - 84.11 µM). Phosphate concentrations in waters of the cenotes and caves are low (not detected - 0.65 µM). Finally, silica concentration ranged from 17.52 to 222.18 µM.

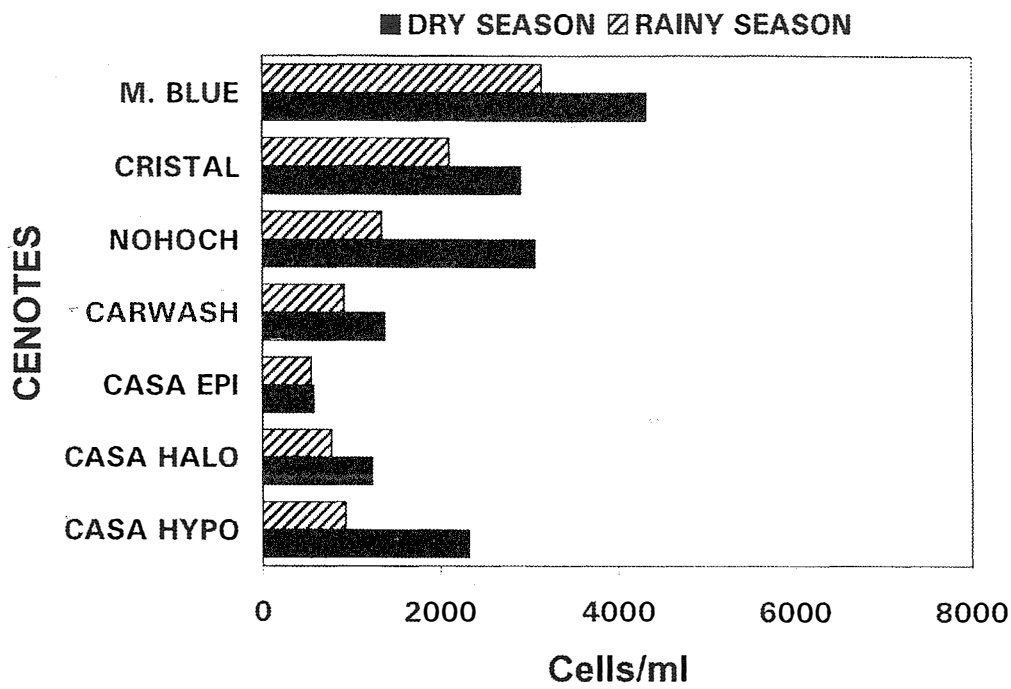

Fig. 1. Seasonal fluctuation of bacterial densities in the studied cenotes. (M. Blue = Mayan Blue cenote, EPI = epicline, HALO = halocline, HYPO = hypocline).

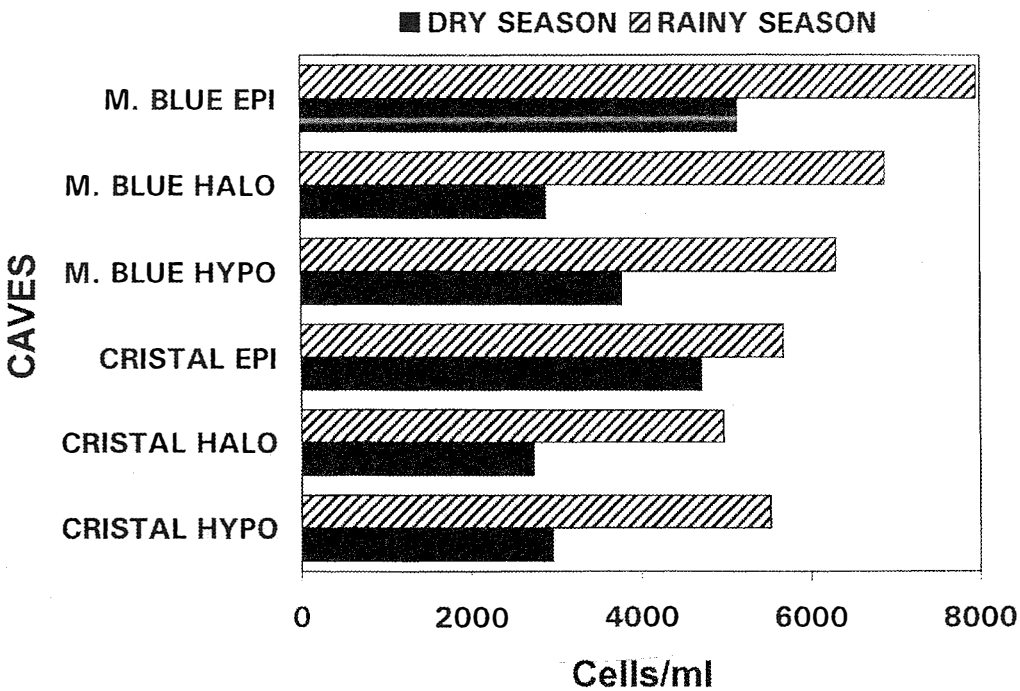

Fig. 2. Seasonal fluctuation of bacterial densities in the studied anchialine caves. (M. Blue = Mayan Blue cave, EPI = epicline, HALO = halocline, HYPO = hypocline).

DISCUSSION

Bacterioplankton densities (in both cenotes and caves) detected in this study are rather low when compared with ordinary densities found in most fresh and marine aquatic ecosystems (Simek *et al.* 1996), which range from 1 to 5 x 10^6 cells/ml. Similar densities (102-104 cells/ml) have been cited from oligotrophic lakes (Ochs *et al.* 1995), and other karst environments (Gounot 1994). However, studies from analogous systems reveal higher densities, such as 10^5 cells/ml in Konza Prairie Research Natural Area, Kansas (Edler & Dodds 1992), and 0.3 - 2.6 x 10^6 cells/ml in the estuary of the Krka river, Croatia (Fuks *et al.* 1994). Our low bacterial densities indicate ultra- to oligotrophic conditions.

The higher bacterial densities found in the caves are probably related to the role of the halocline as a density trap keeping suspended organic matter (plankton and seston) confined to the epicline. Percolation of particulate (POM) as well as dissolved (DOM) organic matter from the forest soil into groundwater could favor bacterial growth, thus increasing bacterial numbers at the epicline.

Ammonia concentration was higher than in other areas of the Yucatan Peninsula (i.e., Herrera-Silveira, 1994, reports 0.1 - 4.2 µM from springs discharging into Celestun lagoon in the state of Yucatan). Nitrites concentrations were similar (0.02 - 15 µM) to other studies in the Yucatan Peninsula (Herrera-Silveira 1994, Herrera-Silveira *et al.* 1998). The range of nitrates is quite similar to that reported by Stoesell *et al.* (1993) for the Mayan Blue cenote. However, nitrate concentrations of the studied cenotes and caves are substantially lower than groundwater nitrate values reported from the state of Yucatan being 1 343 ± 412 µM by Pacheco and Vázquez (1992), 19 - 129 µM by Herrera-Silveira (1994), 65 - 1 129 µM by Marín and Perry (1994), and > 3.2 µM by Pacheco and Cabrera (1997). Nonetheless, large differences in nitrate concentration in waters from adjacent wells in the state of Yucatan suggest, local rather than regional contamination (human and animal organic wastes, organic pesticides, fertilizers, etc.).

The rather low phosphate concentration is due to the presence of high concentrations of calcium -limestone- (Golterman 1984). This condition has been recorded in similar karstic systems elsewhere (Margalef 1983). Comparable phosphate concentrations (0.2 µM) were measured by Stoessel *et al.* (1993) in the Naranjal System, to which the Mayan Blue and Cristal cenotes are related, showing no relevant variation. Silica has been found to be an abundant element (24 - 312 µM) in diverse aquatic ecosystems throughout the Yucatan Peninsula (Herrera-Silveira 1994, Herrera-Silveira *et al.* 1998). Silica concentration in our study was slightly lower than those previously reported for other karstic aquifers.

Nutrients control bacterial production. Vadstein *et al.* (1988) observed bacteria consume phosphates, and a supplement of this compound raises bacterial densities. It is assumed that carbon is the main factor limiting the bacterial growth; however, Edler and Dodds (1996) found no correlation between carbon or phosphorous content to the bacterial densities in karstic aquifers, but they did between nitrogen and bacterial densities. We found no correlation between nitrogen or phosphorous and total bacterial counts.

Edler and Dodds (1996) found a higher number of groundwater bacteria in the Konza Prairie Research Natural Area during the rainy season, than the one we observed in the caves. These authors have associated the high bacterial densities to nitrate input caused by rainwater. However, this is not the case in Quintana Roo, where Alcocer *et al.* (1998) found non-significant differences in nitrate concentrations between the rainy and dry seasons. It seems that, as Gounot (1994) mention, during the rainy season caves are "contaminated" by microorganisms, native to the surface, which are brought in by runoff waters and can develop on exogenous organic matter (animal, human, dead plant fragments, decaying wood, etc.) coming from the cenotes

or the forest soil. It is difficult to know if microorganisms indigenous to the subsurface are present on caves.

Total phosphorous concentrations in our cenotes and caves (0.05 - 1.7 μM) match the interval of ultra- (< 0.064 - 0.419 μM) to oligotrophic (0.064 - 3.2 μM) tropical lakes (Salas & Martino 1988). This trophic category, based on phosphorous, supports the trophic status indicated by bacterial densities, both corresponding to unproductive water bodies (Margalef 1983).

Finally, no chemoautotrophic bacteria were found in the studied anchialine caves. These bacteria are easily recognized since they form macroscopic white-grey colonies growing on the cave floor, cave walls, or even at the water column halocline (Brigmon & Morris 1995). It is quite probable that the presence of dissolved oxygen (1.8 - 2.0 mg/l) throughout the water column (Escobar-Briones *et al.* 1997), even at the halocline, inhibit the growth of this micro-aerophilic or anaerobic bacteria.

ACKNOWLEDGEMENTS

This project was financially supported by Dirección General de Asuntos del Personal Académico, UNAM, project IN203894. The authors would like to thank David Valdés and Elizabeth Real de León (CINVESTAV Unidad Mérida) for carrying out nutrient analyses. Special thanks are given to the Instituto de Ciencias del Mar y Limnología field station Puerto Morelos for providing lodging and laboratory support, and to Mike Madden and his team (especially Chuck Stevens) of CEDAM Dive Center at Puerto Aventuras for providing cave-diving equipment and logistical support. Virginia Urbieta, María Elena García, Laura Peralta, and Luis A. Oseguera are acknowledged for helping in the sampling of biological and water materials.

RESUMEN

Se determinó la fluctuación estacional de las densidades del bacterioplancton en cinco cenotes y dos cuevas anquihalinas del noreste de Quintana Roo, Península de Yucatán. Las densidades del bacterioplancton fueron extremadamente bajas ($5.8 \pm 0.35 \times 10^2$ - $8 \pm 0.48 \times 10^3$ cél/ml). Las densidades bacterianas de los cenotes ($5.8 \pm 0.35 \times 10^2$ - $4.3 \pm 0.26 \times 10^3$ cél/ml) fueron más bajas que en las cuevas ($2.7 \pm 0.16 \times 10^3$ - $8 \pm 0.48 \times 10^3$ cél/ml). El agua de lluvia que se percola a las cuevas a través del suelo de la selva rico en materia orgánica particulada (MOP) y disuelta (MOD), promueve densidades bacterianas más elevadas. Los cenotes y las cuevas se caracterizaron por ser ambientes de ultraoligotróficos a oligotróficos como lo indican las concentraciones bajas de nutrimentos (0.05 - 1.7 μM P total, 34.89 - 250.9 μM N total) así como las densidades bacterianas reducidas.

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